Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 1039–1045
Food Chemistry
www.elsevier.com/locate/foodchem

Comparison of the products of Kombucha fermentation on sucrose and molasses

R. Malbaša *, E. Lončar, M. Djurić

Faculty of Technology, University of Novi Sad, Bul. Cara Lazara 1, 21000 Novi Sad, Serbia

Received 1 February 2007; received in revised form 17 May 2007; accepted 11 July 2007

Abstract

Fermentation of 1.5 g/L of Indian black tea, sweetened either with 70 g/L of sucrose or an adequate quantity of three kinds of molasses, was conducted by domestic Kombucha (*Acetobacter* strains in symbiosis with *Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp. and *Zygosaccharomyces* sp.). Inoculation was performed with 10% of fermentation broth from the previous process. The fermentation in cylindrical vessels containing 2 L of liquid phase, was carried out at 22 ± 1 °C for 14 days, with periodical sampling to measure pH, content of acids (total, acetic and L-lactic), content of remaining sucrose and the yield of biomass at the end of fermentation. Significance of the differences of the results obtained in the fermentations on different substrates was analyzed by applying Duncan's multiple range test. The molasses that yielded the beverage richest in L-lactic acid was suggested for Kombucha fermentation.

Keywords: Kombucha; Molasses; Black tea; L-Lactic acid; Acetic acid

1. Introduction

Sweetened tea (mostly black or green) exposed to Kombucha fermentation gives a pleasantly sour beverage containing a number of compounds which may have a positive effect on human health. Kombucha itself is a symbiotic association of yeasts and bacteria (Dufresne & Farnworth, 2000; Teoh, Heard, & Cox, 2004), whose yeast cells hydrolyze sucrose into glucose and fructose, producing ethanol, while acetic acid bacteria convert glucose to gluconic acid and fructose to acetic acid (Reiss, 1994; Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995). Typical of such fermentation, is the activity of *Acetobacter xylinum* which enables synthesis of a floating cellulose pellicle, in which the embedded cells benefit from the close contact with the atmospheric oxygen (Sievers et al., 1995).

In addition to ethanol and acetic acid, a great number of other compounds emerge as a result of numerous reactions (Balentine, Wiseman, & Bouwens, 1997; Danielova, 1957; Hesseltine, 1965; Kappel & Anken, 1993; Pasha & Reddy, 2005; Petrović & Lončar, 1996; Steiger & Steinegger, 1957). Important metabolites are organic acids – active ingredients of Kombucha tea that may exert beneficial effects (Jayabalan, Marimuthu, & Swaminathan, 2007). Greenwalt, Ledford, and Steinkraus (1998) have confirmed that Kombucha tea has antimicrobial activity against a spectrum of Gram-positive and Gram-negative organisms, the effect being attributed to its acetic acid content. As far as lactic acid is concerned, it appears in Kombucha beverage in its most potent L-form. Malbaša, Lončar, and Kolarov (2002) found that L-lactic acid present in dietetic Kombucha beverages was important for the functioning of the digestive system. Also, lactic acid may be beneficial to blood circulation and contribute to balancing acids and bases in the human body. Finally, it is well known that lactic acid is used as a natural preservative in many food

* Corresponding author. Tel.: +381 21 4853645; fax: +381 21 450413.
E-mail address: bingula@yahoo.com (R. Malbaša).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.07.020

products (Altaf, Naveena, & Reddy, 2007; Wee, Kim, Yun, & Ryu, 2004).

A traditional carbon source for Kombucha fermentation is sucrose. Application of any other sugar (lactose, glucose or fructose) may have a distinct influence on the formation of ethanol and lactic acid, but have only a minor effect on the flavour of the fermented tea (Reiss, 1994). Malbaša (2004) reviewed some attempts in applying atypical nutrients such as Coca-Cola, red wine, white wine, vinegar, extract of Jerusalem artichoke (*Helianthus tuberosus*), milk, fresh sweet whey, reconstituted sweet whey, acid whey, *Echinacea*, *Mentha*, etc. For economic reasons, the use of various agricultural and industrial by-products is also worth considering. Molasses from sugar beet processing, is especially attractive, not only because of its low price but also because of the presence of a number of components including minerals, organic compounds and vitamins, which are very useful for the fermentation process (Rodrigues, Teixeira, & Oliveira, 2006). Molasses is also known as the commonly used carbon source in the industrial production of lactic acid (Kanwar, Tewari, Chadha, Punj, & Sharma, 1995; Shukla et al., 2004; Wee et al., 2004). Our first results on the metabolic activity of Kombucha on sugar beet molasses were published in 2001 (Lončar, Malbaša, & Kolarov, 2001) and, to our knowledge, no other reports on this matter has appeared in the literature so far. The aim of this paper, was to compare the Kombucha fermentation on black tea sweetened with sucrose and to the same extent with three kinds of sugar-beet molasses. The criterion for selecting the most suitable substrate being the highest content of L-lactic acid.

2. Materials and methods

2.1. Kombucha culture

For the fermentation, local domestic Kombucha was used. Primary Kombucha bacterium belongs to the strains of genera *Acetobacter*, while the presence of yeasts *Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp. and *Zygosaccharomyces* sp. was also established (Markov, Malbaša, Hauk, & Cvetković, 2001).

2.2. Molasses characteristics

Molasses samples were obtained from three Serbian sugar factories processing the sugar beet grown in the Pannonian basin. The main characteristics of the molasses (labelled M1, M2 and M3) are presented in Table 1.

2.3. Fermentation systems

The fermentation system with sucrose was prepared in triplicate using 2 L of boiled tap water, 140 g of sucrose (7% solution) and 3 g of black tea (Indian tea, "Vitamin", Horgoš, Serbia). The tea was heated for 5 min at 100 °C, and the leaves were removed by filtration.

The fermentation systems involving selected molasses were prepared (also in triplicate) using 2 L of boiled tap water and a quantity of previously sterilized molasses that contained approximately 140 g of sucrose and 3 g of the same black tea, the further procedure being identical to the previous one.

The prepared liquids were poured into cylindrical vessels of equal size and geometry and, after cooling to room temperature, inoculated with 10% of fermentation broth from the previous Kombucha fermentation. The vessels were covered with cheesecloth and the contents left to incubate at constant temperature of 22 ± 1 °C for 14 days. Samples of the obtained beverages were taken after 0, 3, 7, 10 and 14 days to measure the following parameters: pH, total acids, L-lactic and acetic acid, as well as quantity of the remaining sucrose. Also, the yield of biomass at the end of observation (14th day) was determined.

2.4. Methods of analysis

Values of pH were measured using an electronic pH-meter (Iskra, Kranj, Slovenia). Total acidity was determined by titration with a standard solution of sodium hydroxide and phenolphthalein as indicator.

Acetic acid was determined by UV-method for the determination in foodstuffs and other materials (Böhringer, Mannheim, Cat. No. 148261). This method is based on several enzymatic reactions yielding the formation of NADH. Absorbance was measured at 340 nm.

L-Lactic acid was determined using Böhringer-Mannheim test kit (Cat. No. 139084). L-Lactic acid (L-lactate) was oxidized with NAD in the presence of L-lactate dehydrogenase to pyruvate. By trapping the pyruvate in a subsequent reaction catalyzed by the enzyme glutamate-pyruvate transaminase in the presence of L-glutamate, the equilibrium can be displaced in favour of pyruvate and NADH. The amount of NADH formed is stoichiometric with the amount of L-lactic acid.

Table 1
Characteristics of molasses

Carbon source	Abbrev.	Dry matter (%)	Sucrose (%)	Invert sugar (%)	pH	Total nitrogen (%)	Amino nitrogen (%)	Biotin (μg/100 g)
Molasses from Senta	M1	82.5	49.4	0.95	7.3	1.4	0.25	6.0
Molasses from Kovačica	M2	82.1	47.5	0.73	7.2	1.3	0.18	4.1
Molasses from Srem. Mitrovica	M3	84.2	50.4	0.83	6.7	1.8	0.29	5.5

Sucrose content in the molasses was determined by standard polarimetric method described in the sugar industry manual (Milić, Karadžić, & Obradović, 1992). The sucrose remaining after the fermentation was determined using the Böhringer-Mannheim test kit (Cat. No. 716260).

Yield of the obtained biomass was determined by mass measurement; the cellulose floating pellicle layer was removed from the fermented liquid surface, rinsed with distilled water and dried with filter paper.

3. Results and discussion

3.1. pH values

The decrease of pH values in all systems (Fig. 1) follows the increase in acidity due to the fermentation. The curves are of an exponential character, with the most rapid decay at the beginning of fermentation (to the 3rd day). After the 3rd day, the decrease becomes slower, especially in the systems with molasses M3. As for the systems with molasses M1, their pH values firstly stagnated and then began to rise after the 7th day of fermentation. The slower pH change after the 3rd day may be associated with the buffer effect arising from the reactions between the synthesized organic acids and minerals from the substrate (Lončar, Petrović, Malbaša, & Verac, 2000). The lowest pH values were measured in the systems with molasses M2, a similar pH trend also being observed in the fermentation on sucrose.

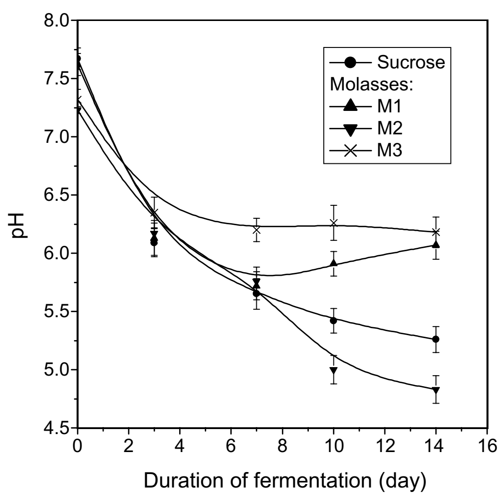

Fig. 1. Values of pH of liquid phase as the functions of process duration on various sources of C-atoms.

The pH values measured at the end of fermentation, appeared to be rather high when compared with either the results of other authors after the fermentation on sucrose (Table 2) or our previous findings for the fermentation on molasses (Table 3). This finding can be related to a lower activity of the applied Kombucha compared to the other cases presented in Tables 2 and 3.

Finally, the pH value can be used as a criterion for ending the fermentation. Experience shows that the acidity of the beverage obtained between 7th and 10th days of fermentation is acceptable from a point of view of sensory characteristics. In our case, 10 days may be suggested as a fermentation period.

3.2. Total acids

Content of total acids as a function of fermentation time is presented in Fig. 2. The starting concentration of acids was higher in all systems with molasses (0.9 g/L in average), compared to the systems with sucrose (0.15 g/L), which can be explained by the presence of free fatty acids in molasses as well as of certain amounts of non-volatile organic acids. Besides, the molasses contained a certain amount of lactic acid from the degradation of invert sugar (Table 1). As the fermentation proceeded, the actual concentration of acids increased in all systems, the increase being smallest in the system with sucrose (from 0.15 g/L to 1.15 g/L). The content of acids in the beverage with sucrose is partly dependent on the metabolic activity of acetic acid bacteria that produce mainly acetic acid. However, the content of total acids in metabolites from the systems with molasses, was about twice as high compared to that in the systems with sucrose. On average, the rise of acidity in the systems with molasses ranged from 0.9 g/L

Table 2
Comparison of relevant published results on sucrose fermentation by Kombucha

Reference	Ferment. conditions		pH	Content of acids, g/L		
	Day	Sucrose, g/L		Total	Acetic	L-Lactic
Petrović et al. (1995/96)	14	70	2.5	6.9	4.1	–[b]
Blanc (1996)	15	70	>2	–[a]	5	>0.6
Lončar et al. (2000)	14	70	2.9	–[b]	–[b]	–[b]
Chen and Liu (2000)	14	95	–[a]	10	8	–[a]
Lončar et al. (2001)	14	70	2.5	8	5.8 (volatile)	–[b]
Malbaša et al. (2002)	14	70	3.1	13.6	5.6	0.3
Lončar et al. (2006)	10	70	3.3	2.7	–[b]	–[b]
Jayabalan et al. (2007)	15	100	3.3	–[a]	6.2	0.33
This paper	14	70	5.3	1.15	0.53	0.05

[a] Not available.
[b] Not tested for the presence.

Table 3
Comparison of relevant published results on molasses fermentation by Kombucha

Reference	Ferment. conditions		pH	Content of acids, g/L		
	Day	Sucrose, g/L		Total	Acetic	L-Lactic
Lončar et al. (2001)	14	70	5.5	3	1.3 (volatile)	–[a]
This paper						
Molasses M1	14	70	6.1	2.5	0.28	0.4
Molasses M2	14	70	4.8	4.2	0.24	0.16
Molasses M3	14	70	6.2	2.5	0.21	0.32

[a] Not tested for the presence.

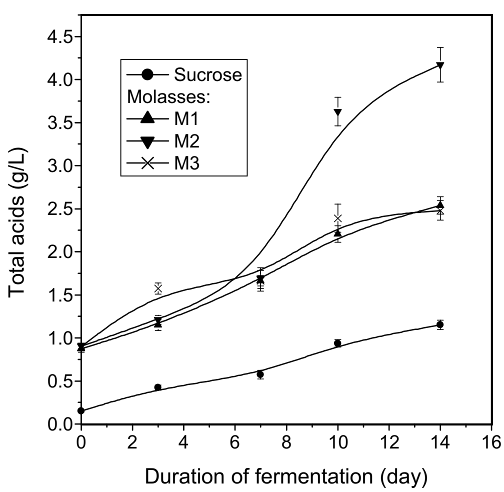

Fig. 2. Content of total acids in liquid phase as the function of process duration on various sources of C-atoms.

to 3.1 g/L, the most significant being in the case of molasses M2 (from 0.9 g/L to 4.2 g/L). The higher content of total acids in the molasses fermentation samples is due to the chemical composition of molasses, which make a very convenient substrate for the industrial manufacturing of different organic acids such as lactic, butyric, propionic, acetic, citric, fumaric and gluconic (Došenović, 2004).

The significance of the differences between total acidities of the investigated systems was determined by applying Duncan's multiple range test (Table 4). It showed that total acidity of the product of sucrose fermentation was significantly smaller compared to all the systems with molasses. On the other hand, no difference between the systems with molasses M1 and M3 was observed. Comparison of the systems with molasses M1 and M2 as well as M2 and M3 showed that the differences were insignificant for the first 7 days and became significant afterwards.

Total acidities measured in this investigation were rather low compared to the results previously reported for the fermentation on sucrose (Table 2) and molasses (Table 3), which can be ascribed to the quite low activity of the applied Kombucha. Finally, if the total acidity of fermented tea is related to its pH value, it may be concluded that there is no a direct correlation between them. As was also noticed by Lončar et al. (2001), the tea sweetened with sucrose had a lower pH than the tea with molasses, although the former had a lower content of total acids. This means that the fermented tea with molasses will have milder sour taste but higher content of acids.

3.3. Acetic acid

The changes of acetic acid content as a function of the process duration in the systems with various carbon sources are shown in Fig. 3. When sucrose was used as carbon source, acetic acid was one of the main metabolites of the acetic acid bacteria from Kombucha association. The growth of bacteria, was especially intensive during the early stage of the fermentation. After 14 days of fermentation on sucrose, the concentration of acetic acid amounted to 0.53 g/L, which was 50% of all acids. However, on the substrates with molasses, the acetic acid content made only one half of that obtained on sucrose in the same phase of

Table 4
Significances of the differences between contents of total acids (ta), acetic acid (aa) and lactic acid (la) in liquid phase during fermentation on various carbon sources

Ferm.	Sucrose and molasses									Molasses								
	S&M1			S&M2			S&M3			M1&M2			M1&M3			M2&M3		
(day)	ta	aa	la	ta	aa	la	ta	aa	la	ta	aa	la	ta	aa	la	ta	aa	la
0	+[a]	+	+	+	+	+	+	+	+	–[b]	+	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	–
7	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–	–	–	+
10	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	–	+
14	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+

[a] + the difference is significant.
[b] – the difference is not significant.

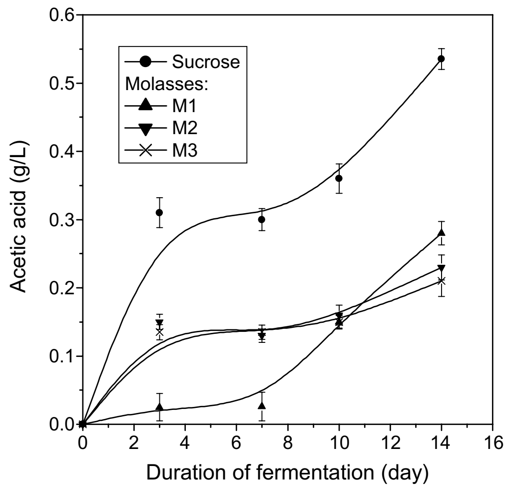

Fig. 3. Content of acetic acid in liquid phase as the function of process duration on various sources of C-atoms.

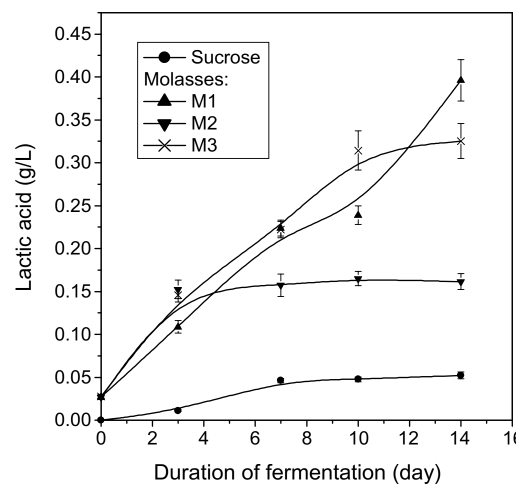

Fig. 4. Content of L-lactic acid in liquid phase as the function of process duration on various sources of C-atoms.

fermentation. It might be speculated that, due to the presence of some components from the molasses, either the activity of acetic acid bacteria decreased or the generated acetic acid was converted into other compounds. The rise of acetic acid content was particularly slow in the case of molasses M1. However, at the end of observation, the concentrations of acetic acid in all systems with molasses became similar (from 0.21 g/L on M3 to 0.28 g/L on M1).

The application of Duncan's multiple range test (Table 4) showed that the product of sucrose fermentation contained a significantly greater quantity of acetic acid, compared to all the systems with molasses. Smaller, but still significant differences of this parameter were also found between the systems with molasses M1 and M2 as well as M1 and M3. No significant differences were observed between the systems with molasses M2 and M3.

Contents of acetic acid measured in this investigation appeared to be very low compared with the results of fermentation on both sucrose (Table 2) and molasses (Table 3). This can be explained in terms of a low activity of the acetic acid bacteria of Kombucha association in converting fructose to acetic acid.

3.4. L-Lactic acid

Changes of the content of L-lactic acid with the duration of fermentation on different carbon sources are presented in Fig. 4. As can be seen, a very small increase in concentration of L-lactic acid was obtained in the fermentation on sucrose (from 0 g/L to 0.053 g/L). On the contrary, metabolites obtained on molasses contained much larger amounts of L-lactic acid, the descending sequence being M1 > M3 > M2. This sequence coincides with the sequence of the contents of invert sugar in the molasses (0.95% in M1, 0.83% in M3 and 0.73% in M2). The increase in L-lactic acid was more significant in the early phase of fermentation (up to the 3rd day) on all molasses. After the 3rd day, the content of L-lactic acid in the system with molasses M2 remained constant (0.16 g/L) to the end of the process, whereas in the systems with M1 and M3 the increasing trend continued, reaching 0.4 g/L (on M1) and 0.36 g/L (on M3).

Statistical analysis of the differences between the contents of L-lactic acid in the investigated systems was performed by applying Duncan's multiple range test (Table 4). It appeared that the content of L-lactic acid was significantly smaller in the system with sucrose compared to all the systems with molasses. The differences between the systems with molasses were also significant, the smallest difference being between M1 and M3 as substrate.

The contents of L-lactic acid after the fermentation on molasses M1, M2 and M3 were higher compared to those achieved on sucrose (Table 2), despite a lower activity of the Kombucha used in this work. This fact, which undoubtedly should be considered as an advantage, can be explained in several ways. As mentioned above, molasses contain certain amounts of lactic acid resulting from the degradation of invert sugar. In addition, as the molasses contain the growing factor biotin and amino nitrogen (Table 1), they are advantageous as a fermentation substrate over pure sucrose, and this could be the reason for a more intensive Kombucha metabolism, hence resulting in higher contents of L-lactic acid. As is well known, glycolysis is the first anaerobic step in the metabolism of glucose, whose end products are pyruvate and lactate. In the cytosolic phase of glycolysis, excess pyruvate is converted to lactate by the enzyme lactate dehydrogenase. The decline of the availability of oxygen causes pyruvate accumulation, resulting in the conversion of pyruvate to lactate. The production of lactate has an important role in anaerobic respiration. The conversion of glucose to pyruvate requires NAD^+ to act as a substrate. This is produced from the oxidation of NADH by the electron transport chain. During oxygen deficiency, the electron transport chain cannot function as there is no ultimate electron acceptor, so that the process of regenerating of NAD^+ ceases, and glycolysis cannot proceed because of the lack of substrate (Handy,

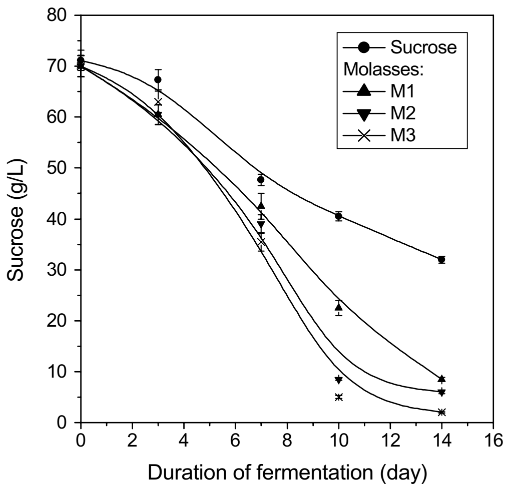

Fig. 5. Content of sucrose in liquid phase as the function of process duration on various sources of C-atoms.

2006). As the Kombucha is an association of aerobic bacteria and facultative anaerobic yeasts, the system itself ferments without forced oxygenation, so that the produced lactic acid can possibly serve as an electron sink.

As the aim of this investigation was to produce fermented tea richest in L-lactic acid, molasses M1 and M3, having highest contents of invert sugar, biotin and amino nitrogen (Table 1), can be suggested as a carbon source.

3.5. Remaining sucrose

By the enzyme invertase, Kombucha converts sucrose to glucose and fructose and further to ethanol, acetic acid, and a large number of other compounds. Therefore, the time decrease of sucrose concentration (Fig. 5) may be used as a measure of the rate of fermentation. At the beginning, the process in all the systems, containing approximately 70 g/L of sucrose, was rather slow. After the 3rd day, the sucrose consumption began to accelerate and continued to do so until the 10th day, and then to slow down by the end of observation. The obtained results are in agreement with the published data (Sievers et al., 1995). The decay of sucrose concentration in the systems with molasses was much faster than in the systems with pure sucrose. It was most rapid in the case of molasses M3 – the sucrose being consumed almost completely to the end of fermentation.

3.6. Yield of biomass

The yield of biomass increased during the fermentation in dependence of the nature of the substrate. The smallest quantity was found for the system with sucrose (17.1 g), nine times larger in the case of molasses M1 (154.8 g), and almost 10 times larger in the case of molasses M2 (165.6 g). Kombucha mass was the largest after 14 days of fermentation on molasses M3 (270.8 g). As the amounts of biomass in particular systems differed so significantly, the differences could be seen even with the naked eye. Such observation may be related to the significant content of total nitrogen in the systems with molasses. Namely, the only source of nitrogen during the fermentation on sucrose is tea, while molasses itself may contain non-sucrose organic matter at a level of about 20% of total mass. Here, content of total nitrogen, including nitrogen from free amino acids, pyrrolidone carboxylic acid, peptides, nucleic acid components, etc. can be up to 2% of total mass, or even slightly higher (Došenović, 2004). As the content of nitrogen-containing compounds in the systems with molasses was much higher compared to that with sucrose, the observed differences in the biomass yield must be ascribed to these components; the highest content of total nitrogen in M3 (Table 1) correlates with the highest yield of Kombucha biomass.

3.7. Sensory characteristics of fermented tea

Black tea fermented by Kombucha is usually light brown coloured, sparkling, sour and refreshing beverage. Kombucha beverages obtained on molasses were dark, slightly carbonated and sweet, with caramelized taste and smell typical of molasses.

4. Conclusion

The characteristics of the main products of black tea fermentation by Kombucha on pure sucrose and three kinds of molasses were compared, and the following conclusions were drawn: the pH values were lower in the system with sucrose; the total acidity was higher in the systems with molasses; the content of acetic acid was higher in the systems with sucrose; the content of L-lactic acid was significantly larger in the systems with molasses, particularly in the systems with molasses M1 and M3; the content of sucrose decreased faster in the systems with molasses; the yield of biomass was significantly larger with molasses.

This investigation has confirmed that the molasses from sugar beet processing can be used as a low-cost carbon source in Kombucha fermentation of black tea. The products obtained on these substrates were rich in lactic acid, which may be considered as an advantage compared to the product on sucrose. The content of lactic acid is related to the higher quantity of invert sugar, biotin and amino nitrogen in the molasses.

Acknowledgement

Financial support from the Ministry of Science and Environmental Protection of Serbia is highly acknowledged (Grant BTN-371002B).

References

Altaf, Md., Naveena, B. J., & Reddy, G. (2007). Use of inexpensive nitrogen sources and starch for L(+) lactic acid production in anaerobic submerged fermentation. *Bioresource Technology, 98*, 498–503.

Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Critical Review in Food Science and Nutrition, 37*, 693–704.

Blanc, Ph. (1996). Characterization of tea fungus metabolites. *Biotechnology Letters, 18*, 139–142.

Chen, C., & Liu, B. Y. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of Applied Microbiology, 89*, 834–839.

Danielova, L. T. (1957). K khimicheskomu sostavu i fiziko-khemicheskim svoistvam kulturalnoi zhidkosti chainogo gryba. *Trudy Erevanskogo zooveterinarnogo Instituta, 22*, 111–121.

Došenović, I. (2004). Development of methods for biotin determination in molasses. Ph.D. Thesis, University of Novi Sad, Faculty of Technology, Novi Sad.

Dufresne, C., & Farnworth, E. (2000). Tea, kombucha and health: A review. *Food Research International, 33*, 409–421.

Greenwalt, C. J., Ledford, R. A., & Steinkraus, K. H. (1998). Determination and characterization of the antimicrobial activity of the fermented tea kombucha. *Lebensmittel Wissenschaft und Technologie, 31*, 291–296.

Handy, J. (2006). Lactate – The bad boy of metabolism or simply misunderstood? *Anaesthesia & Critical Care, 17*, 71–76.

Hesseltine, C. W. (1965). A millenium of fungi. Food and fermentation. *Mycologia, 57*(2), 148–167.

Jayabalan, R., Marimuthu, S., & Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chemistry, 102*, 392–398.

Kanwar, S. S., Tewari, H. K., Chadha, B. S., Punj, V., & Sharma, V. K. (1995). Lactic acid production from molasses by *Sporolactobacillus cellulosolvens*. *Acta Microbiologica and Immunologica Hungarica, 42*, 331–338.

Kappel, T., & Anken, R. H. (1993). The tea mushroom. *The Mycologist, 7*, 12–13.

Lončar, E., Djurić, M., Malbaša, R., Kolarov, Lj., & Klašnja, M. (2006). Influence of working conditions upon Kombucha conducted fermentation of black tea. *Food and Bioproducts Processing, 84*(C3), 186–192.

Lončar, E. S., Malbaša, R. V., & Kolarov, Lj. A. (2001). Metabolic activity of tea fungus on molasses as a source of carbon. *Acta Periodica Technologica, 32*, 21–26.

Lončar, E. S., Petrović, S., Malbaša, R. V., & Verac, R. M. (2000). Biosynthesis of glucuronic acid by means of tea fungus. *Nahrung, 44*, 138–139.

Malbaša, R.V. (2004). Investigation of antioxidant activity of beverage from tea fungus fermentation. Ph.D. Thesis, University of Novi Sad, Faculty of Technology, Novi Sad.

Malbaša, R., Lončar, E., & Kolarov, Lj. (2002). L-Lactic, L-ascorbic, total and volatile acids contents in dietetic kombucha beverage. *Roumanian Biotechnological Letters, 7*(5), 891–895.

Markov, S. L., Malbaša, R. V., Hauk, M. J., & Cvetković, D. D. (2001). Investigation of tea fungus associations. I. The Yeasts. *Acta Periodica Technologica, 32*, 133–138.

Milić, M., Karadžić, V., & Obradović, S. (1992). *Methods for laboratory control of sugar production in a factory*. Novi Sad: University of Novi Sad, Institute for Sugar.

Pasha, C., & Reddy, G. (2005). Nutritional and medicinal improvement of black tea by yeast fermentation. *Food Chemistry, 89*, 449–453.

Petrović, S., & Lončar, E. (1996). Content of water-soluble vitamins in fermentative liquids of tea fungus. *Mikrobiologija, 33*, 101–106.

Petrović, S., Lončar, E., Ružić, N., & Kolarov, Lj. (1995/96). Nutritive characteristics of tea fungus metabolites. *Acta Periodica Technologica*(26/27), 257–269.

Reiss, J. (1994). Influence of different sugars on the metabolism of the tea fungus. *Zeitschrift fur Lebensmittel Untersuchung und Forschung, 198*, 258–261.

Rodrigues, L. R., Teixeira, J. A., & Oliveira, R. (2006). Low-cost fermentative medium for biosurfactant production by probiotic bacteria. *Biochemical Engineering Journal, 32*, 135–142.

Shukla, V. B., Zhou, S., Yomano, L. P., Shanmugam, K. T., Preston, J. F., & Ingram, L. O. (2004). Production of D(−)-lactate from sucrose and molasses. *Biotechnology Letters, 26*, 689–693.

Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., & Teuber, M. (1995). Microbiology and fermentation balance in kombucha beverage obtained from a tea fungus fermentation. *Systems Applied Microbiology, 18*, 590–594.

Steiger, K. E., & Steinegger, E. (1957). Uber den Teepilz. *Pharmaceutica Acta Helvetica, 32*, 133–154.

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of kombucha fermentation. *International Journal of Food and Microbiology, 95*, 119–126.

Wee, Y., Kim, J., Yun, J., & Ryu, H. (2004). Utilization of sugar molasses for economical L(+)-lactic acid production by batch fermentation of Enterococcus faecalis. *Enzyme and Microbial Technology, 35*, 568–573.